SECU

AD-A193 138

ENTATION PAGE

Form Approved OMB No. 0704-0188

1a REPORT SECURITY CLASSIFICATION: (U)	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY: NA	3. DISTRIBUTION/AVAILABILITY OF REPORT: Distribution Unlimited
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE: NA	
4 PERFORMING ORGANIZATION REPORT NUMBER(S): University of Wisconsin	5. MONITORING ORGANIZATION REPORT NUMBER(S): NA
6a. NAME OF PERFORMING ORGANIZATION: University of Wisconsin; 6b. OFFICE SYMBOL (If applicable): NA	7a. NAME OF MONITORING ORGANIZATION: Office of Naval Research
6c. ADDRESS (City, State, and ZIP Code): Department of Anesthesiology, 600 Highland Avenue B6/387, Madison, WI 53792	7b. ADDRESS (City, State, and ZIP Code): 800 North Quincy Street, Arlington, VA 22217-5000
8a. NAME OF FUNDING/SPONSORING ORGANIZATION: Office of Naval Research; 8b OFFICE SYMBOL (If applicable): ONR	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER: N00014-86-K-0490
8c. ADDRESS (City, State, and ZIP Code): 800 North Quincy Street, Arlington, VA 22217-5000	10 SOURCE OF FUNDING NUMBERS: PROGRAM ELEMENT NO. 61153N; PROJECT NO. RR04108; TASK NO. 4414 449; WORK UNIT ACCESSION NO.

11. TITLE (Include Security Classification)
Pharmacologic Studies on the In Vitro Bronchodilating/Vasoactive Actions of Oligo-PGB.

12. PERSONAL AUTHOR(S)
Saban, Ricardo; Li, Nian-Ji and Will, James A.

13a. TYPE OF REPORT	13b TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Final	FROM 8/1/86 TO 12/31/87	January 6, 1988	16

16 SUPPLEMENTARY NOTATION

17 COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	Oligo-PGB, bronchodilatation, hypersensitive response.
08			

19 ABSTRACT (Continue on reverse if necessary and identify by block number)

Oligomeric mixtures of prostaglandin B_1 (O-PGB) relaxes airways of guinea pig when studied in vitro. Bronchi with the epithelium preserved or removed were exposed to contractile agent for 20 minutes either in presence or in absence of indomethacin $5x10^{-6}M$. O-PGB was added at 10^{-6} and $10^{-5}M$ when present, and the results were defined in terms of percent of maximum relaxation induced by papaverine $10^{-3}M$. Substance P (SP) $3x10^{-8}M$, leukotriene D4 (LTD4) $1x10^{-9}M$ and carbachol $1x10^{-8}M$ induced similar contractions. The degree of relaxation induced by O-PGB was dependent upon the contractil agoinst (36% of papaverine when the bronchi were precontrated with SP, 25%-against LTD4 and only 15% against carbachol) suggesting that the mechanism is specific. The relaxation was not altered by removing the epithelial cells or by the addition of indomethacin, therefore, the effect of O-PGB seems to occur independently of cyclooxygenase-or (over)

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT ☐ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS	21. ABSTRACT SECURITY CLASSIFICATION
22a NAME OF RESPONSIBLE INDIVIDUAL	22b TELEPHONE (Include Area Code) / 22c OFFICE SYMBOL

DD Form 1473, JUN 86 — Previous editions are obsolete. — SECURITY CLASSIFICATION OF THIS PAGE

19. (Continued)

or epithelium- derived substances. We also analysed the effect of O-PGB on "acute" and "chronic" preparations in regard to immediate hypersensitivet phenomenon. Animals were actively sensitized to ovalbumin (OVA) 3 weeks prior to testing to OVA in vitro. I \ "acute" experiments airways were exposed to OVA in vitro with or without the presence of O-PGB at 10^{-5}M, followed by a maximal contraction to $BaCl_2$, $3x10^{-2}$M. No difference was found. "Chronic" experiments with sensitized animals were carried out in airway segments incubated for 18 or 24 hours in RPMI 1640 medium with or without O-PGB. The contractil response were identical after 18 hours. We found that bronchi incubated for 24 hours with O-PGB showed a significant increase in OVA-induced contractions. Indomethacin $5x10^{-6}$M added to the tissue bath enhanced the control dcse-response curve but failed to further increase the responses of O-PGB-treated tissues. These experiments seem to show that O-PGB has two distinct effects: 1) acutely it relaxes precontract airways in a agonist-dependent manner and 2) increases the hypersensitivity responses of bronchi isolated from sensitized animals, when incubated 24 hours.

Final Report on Contract N00014-86-K-0490

Principal Investigator: James A. Will, D.V.M., Ph.D.

Contractor: Board of Regents, University of Wisconsin-Madison

Contract Title: Pharmacologic Studies on the *In Vitro* Bronchodilating Vasoactive Actions of Oligo-PGB

Start Date: 1 August, 1986

Research Objective: To determine if oligo-PGB has effects on the smooth muscle of airway and vasculature of guinea pigs and humans studied in vitro.

We have determined that oligo-prostaglandin B (oligo-PGB) relaxes airways of guinea pigs when studied in vitro. In this study we used both high molecular weight mixture (HMW) and oligo-PGB forms of the compound. We also investigated whether or not the relaxation is dependent upon the concentration of oligo-PGB, the contractile substance used to enhance tone, the integrity of epithelial cells layer and the release of cyclooxygenase metabolites. We further analysed the effect of oligo-PGB on "acute" (protocol IV) and "chronic" (protocol V) preparations in regard to the immediate hypersensitivity phenomenon. In the chronic preparation, the tissue was incubated in the presence of oligo-PGB for 18 or 24 hours.

In protocol I, we either tested HMW and oligo-PGB in the absence of a contractile agent or after exposing the tissue to carbachol (Cch) for 20 minutes. The studies were further differentiated by doing the experiments either in the presence or absence of indomethacin, a prostaglandin synthesis inhibitor at 5 times 10^{-6} M and the results are defined in terms of percent of maximum relaxation induced by papaverine added at 10^{-3}M.

We compared the activity of HMW with that of oligo-PGB. HMW relaxed to a lesser degree than oligo-PGB at 10^{-6}M, but caused slight contractions at 10^{-5}M when no agent was used to precontract the airway (bronchi). This protocol relies on the inherent tone of the tissues. When airways were precontracted with carbachol (Cch) at $3X10^{-8}$M, the results were similar.

We next attempted to determine the optimal conditions for the relaxation. From this point we did all experiments with the trimer, oligo-PGB. When

tissues were precontracted with Cch, there appeared to be a trend toward decreased relaxation with a decrease in extracellular Ca^{++} from 2.5 to 0.5 mM. The level of contraction with Cch was unaltered in a low Ca^{++} substrate.

In protocol II, tissues with the epithelium preserved or removed were exposed to the contractile agent for 20 minutes either in presence or in the absence of indomethacin ($5X10^{-6}$M). oligo-PGB was added at 10^{-6} (group A) and 10^{-5}M (group B) When bronchi were precontracted with substance P (SP), or leukotriene D_4 (LTD_4) the magnitude of contraction was similar to that of Cch. SP-precontracted tissues relaxed more than LTD_4 or Cch (figure 1 and table 1). In SP and LTD_4-precontracted tissues there was a tendency for a dose-dependent relaxation (figure 1 and table 1). There were no differences between the relaxation responses with Cch precontracted tissues at either 10^{-5} or 10^{-6}M with the exception of the group with intact epithelial cells and in the presence of indomethacin which showed a greater degree of relaxation at 10^{-5}M than at 10^{-6}M of oligo-PGB. We suggest that the complete dose-response curve to oligo-PGB will be the best protocol to study this relationship.

To exclude the possiblity that the oligo-PGB relaxation could be due to a release of an inhibitory prostaglandin, we blocked production of prostaglandins by pretreating the tissues with indomethacin. Pretreatment did not affect the amount of tone induced by the different contractile agonists nor the magnitude of relaxation induced by oligo-PGB.

We also considered the possibility that bronchial epithelial cells could interact with the activity of oligo-PGB. We tested this hypothesis by mechanically removing the epithelial cells of the bronchi. This procedure did not alter the activity of oligo-PGB, wtih the exception of the tissues precontracted with Cch in the presence of indomethacin where oligo-PGB at 10^{-6}M elicited twice as much relaxation in tissues with the epithelial cells removed than with the matched control (Figure 1 and table 1). We are unable to explain these differences. It appears that an epithelium-derived factor does not participate in the relaxation induced by oligo-PGB.

In protocol III, dose response curves to SP and LTD_4 were obtained in the presence or absence of oligo-PGB added 20 minutes before the compound in study. oligo-PGB at 10^{-5} failed to exhibit any antagonism of SP (figure 2) or LTD_4 (figure 3) contractile responses.

Protocols IV and V, were designed to determine if oligo-PGB plays a role in the immediate hypersensitivity response. Animals were actively

sensitized to ovalbumin (OVA) 3 weeks prior to testing. In "acute" experiments (protocol IV), airways were exposed to OVA *in vitro* with or without the presence of oligo-PGB at 10^{-5}M, followed by a maximal contraction to $BaCl_2$ ($3X10^{-2}$M). No difference was found (figure 4).

"Chronic" experiments (Protocol V) with sensitized animals were carried out in airway segments incubated for: 18 (overnight) or 24 hours in RPMI 1640 media (Sigma Chemical, Co.) with or without oligo-PGB and then dose response curves to OVA were run in the presence or absence of oligo-PGB and compared to the maximal contraction by $BaCl_2$. The contractile responses to OVA were identical after 18 nours of incubation with or without oligo-PGB (figure 5). In contrast, we found that in bronchi incubated for 24 hours with oligo-PGB, the contractions to OVA increased (figure 6). While 18 hours had no effect, and the fact that 24 hours showed a significant increase in contraction would imply that there is a critical time of exposure to oligo-PGB in order to cause these differences. This does not appear to be reasonable and we need to increase the number of experiments with 18 hours of incubation. In our previous progress report, only the 18 hours incubation information was included. Indomethacin ($5X10^{-6}$M) added to the bath of tissues incubated for 24 hours enhanced the responses of control but failed to further increase the responses of O-PGB-treated tissues (figure 7).

We also examined the dose response curve to OVA in the presence of pyrilamine (histamine H_1-receptor antagonist) alone or in combination with indomethacin. Pyrilamine reduced the response of tissues incubated for 24 hours to OVA, regardless of the presence or absence of oligo-PGB in the incubation medium (figure 8). The combination of indomethacin and pyrilamine abolished the differences between control and O-PGB-treated tissues (figure 9).

These experiments seem to show that oligo-PGB has two distinct effects:

1) Acutely it relaxes precontracted airways in an agonist-dependent manner.

2) oligo-PGB increases the hypersensitivity responses of bronchi isolated from sensitized animals, when incubated for 24 hours.

The conclusions of these experiments are:

1) oligo-PGB relaxes airways.

2) Relaxation by oligo-PGB is dependent upon the contractile agonist; suggesting that the mechanism is specific.

3) Relaxation by oligo-PGB occurs independently of cyclooxygenase- or epithelium-derived substances.

4) Treatment with oligo-PGB may alter the immediate hypersensitivity phenomenon after 24 hours implying that the alteration requires time to occur. Although the mechanism by which oligo-PGB increases the responses to OVA is not known, the experiments with indomethacin indicate that oligo-PGB might have some effect on arachidonic acid metabolism.

Legend Figure 1.

Relaxation induced by oligo-PGB in guinea pig isolated bronchi expressed as percent of the maximum induced by papaverine ($1X10^{-3}M$). Tissues were contracted with substance P ($3X10^{-8}M$), LTD_4 ($1X10^{-9}M$) or carbachol ($1X10^{-8}M$). The bronchi had the epithelium preserved or mechanically removed. Indomethacin ($5X10^{-6}M$) was presented for 2 hours, where indicated. Letters of each column corresponded to "t" test of table 1. N=8 for all groups, except for: group K (N=9), S (N=6), U (N=6) and Z (N=10).

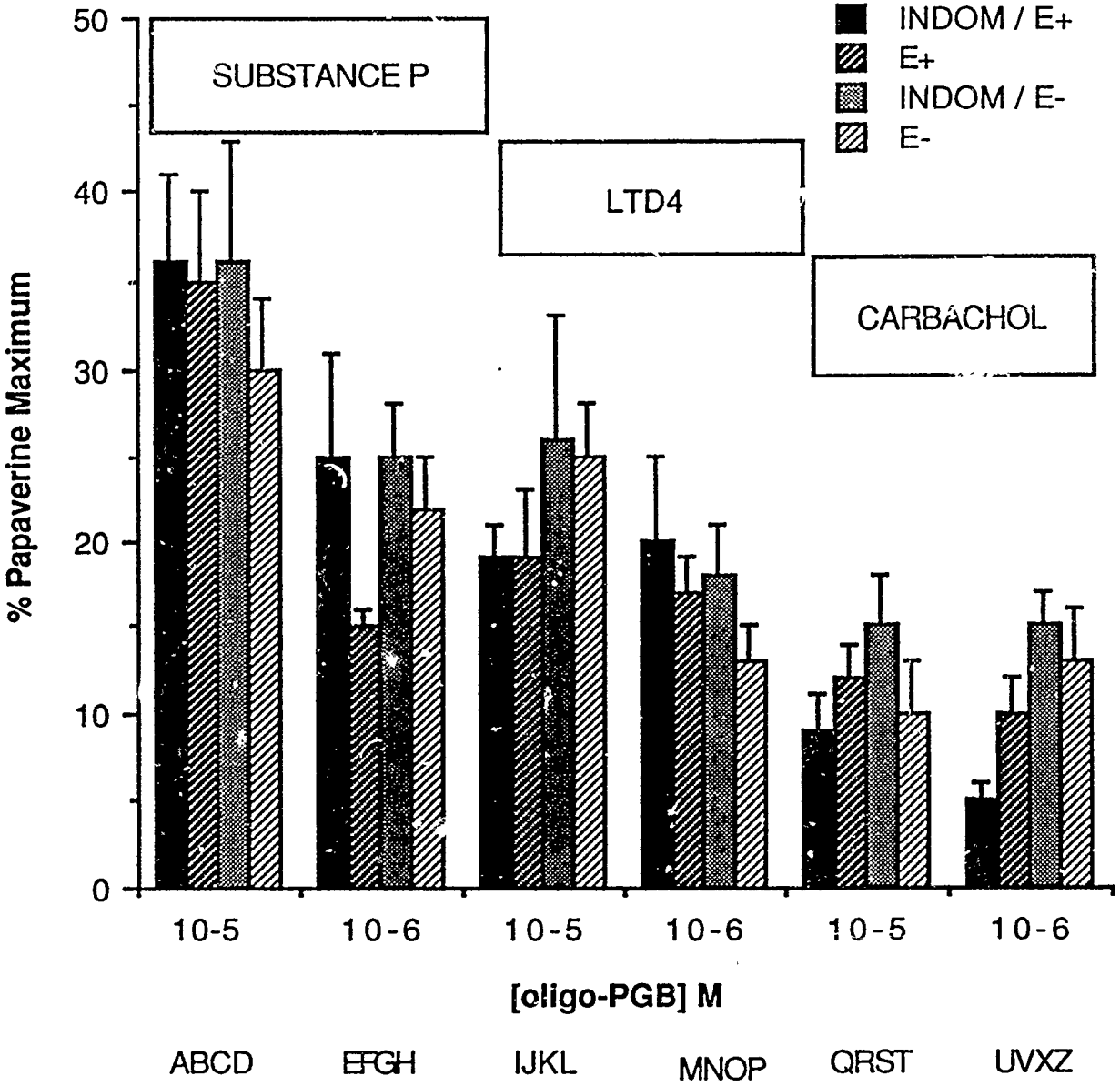
50
40
30
20
10
0
% Papaverine Maximum
SUBSTANCE P
LTD4
CARBACHOL
INDOM / E+
E+
INDOM / E-
E-
10-5
10-6
10-5
10-6
10-5
10-6
[oligo-PGB] M
ABCD
EFGH
IJKL
MNOP
QRST
UVXZ

FIG.1

ANALYSIS	[oligo-PGB]	TREATMENT	COLUMN FIG.1	D.F.	t VALUE
SP X LTD4	5	E+ / INDO	A / I	14	3.19*
SP X LTD4	5	E+	B / J	14	2.50*
SP X LTD4	5	E- / INDO	C / K	14	1.01
SP X LTD4	5	E -	D / L	14	0.87
SP X CCH	5	E+ / INDO	A / Q	14	4.10*
SP X CCH	5	E+	B / R	14	4.13*
SP X CCH	5	E- / INDO	C / S	15	2.83*
SP X CCH	5	E -	D / T	14	4.11*
SP X LTD4	6	E+ / INDO	E / M	14	0.72
SP X LTD4	6	E+	F / N	14	0.05
SP X LTD4	6	E-	G / O	12	1.53
SP X LTD4	6	E- / INDO	H / P	14	2.17
SP X CCH	6	E+ / INDO	E / U	14	3.40*
SP X CCH	6	E+	F / V	14	2.83*
SP X CCH	6	E-/ INDO	G / X	14	2.30*
SP X CCH	6	E -	H / Z	16	1.84
LTD4 X CCH	5	E+ / INDO	I / Q	14	3.39*
LTD4 X CCH	5	E+	J / R	14	1.3
LTD4 X CCH	5	E- / INDO	K / S	15	1.09
LTD4 X CCH	5	E -	L / T	14	3.79*
LTD4 X CCH	6	E+ / INDO	M / U	14	2.96*
LTD4 X CCH	6	E+	N / V	14	2.25*
LTD4 X CCH	6	E- / INDO	O / X	12	0.59
LTD4 X CCH	6	E -	P / Z	16	0.11
SP +INDO / -INDO	5	E+	A / B	14	0.15
+INDO / -INDO	5	E -	C / D	14	0.74
+INDO / -INDO	6	E+	E / F	14	1.41
+INDO / -INDO	6	E -	D / H	14	0.56
SP E+ / E-	5	INDO	A / C	14	0.01
E+ / E-	5		B / D	14	0.72
E+ / E-	6	INDO	E / G	14	0.12
E+ / E-	6		F / H	14	1.25
SP 10-5 / 10-6		E+ / INDO	A / E	14	1.38
10-5 / 10-6		E+	B / F	14	3.58*
10-5 / 10-6		E- / INDO	C / G	14	1 56
10-5 / 10-6		E -	D / H	14	1.48

TABLE 1. STATISTICAL ANALYSIS OF THE DATA FROM FIGURE 1 * indicates p<0.05

LTD4					
+INDO /-INDO	5	E+	I / J	14	0.04
+INDO /-INDO	5	E -	K / L	14	0.11
+INDO /-INDO	6	E+	M / N	14	0.53
+INDO /-INDO	6	E -	O / P	12	1.37
LTD4					
E+ / E-	5	INDO	I / K	14	1.03
E+ / E-	5		J / L	14	1.2
E+ / E-	6	INDO	M / O	12	0.37
E+ / E-	6		N / P	14	1.43
LTD4					
10-5 / 10-6		E+ / INDO	I / M	14	1.03
10-5 / 10-6		E+	J / N	14	0.35
10-5 / 10-6		E- / INDO	K / O	12	1.03
10-5 / 10-6		E -	L / P	14	3.11*
OOH					
+INDO / -INDO	5	E+	Q / R	14	1.21
+INDO / -INDO	5	E -	S / T	15	1.26
+INDO / -INDO	6	E+	U / V	14	1.95
+INDO / -INDO	6	E -	X / Z	16	0.66
OOH					
E+ / E-	5	INDO	Q / S	15	1.73
E+ / E-	5		R / T	14	0.67
E+ / E-	6	INDO	U / X	14	4.17
E+ / E-	6		V / Z	16	0.59
OOH					
10-5 / 10-6		E+ / INDO	Q / U	14	1.43
10-5 / 10-6		E+	R / V	14	0.67
10-5 / 10-6		E- / INDO	S / X	15	0.73
10-5 / 10-6		E -	T / Z	16	0.57

TABLE 1. STATISTICAL ANALYSIS OF THE DATA FROM FIGURE 1. * indicates $p < 0.05$

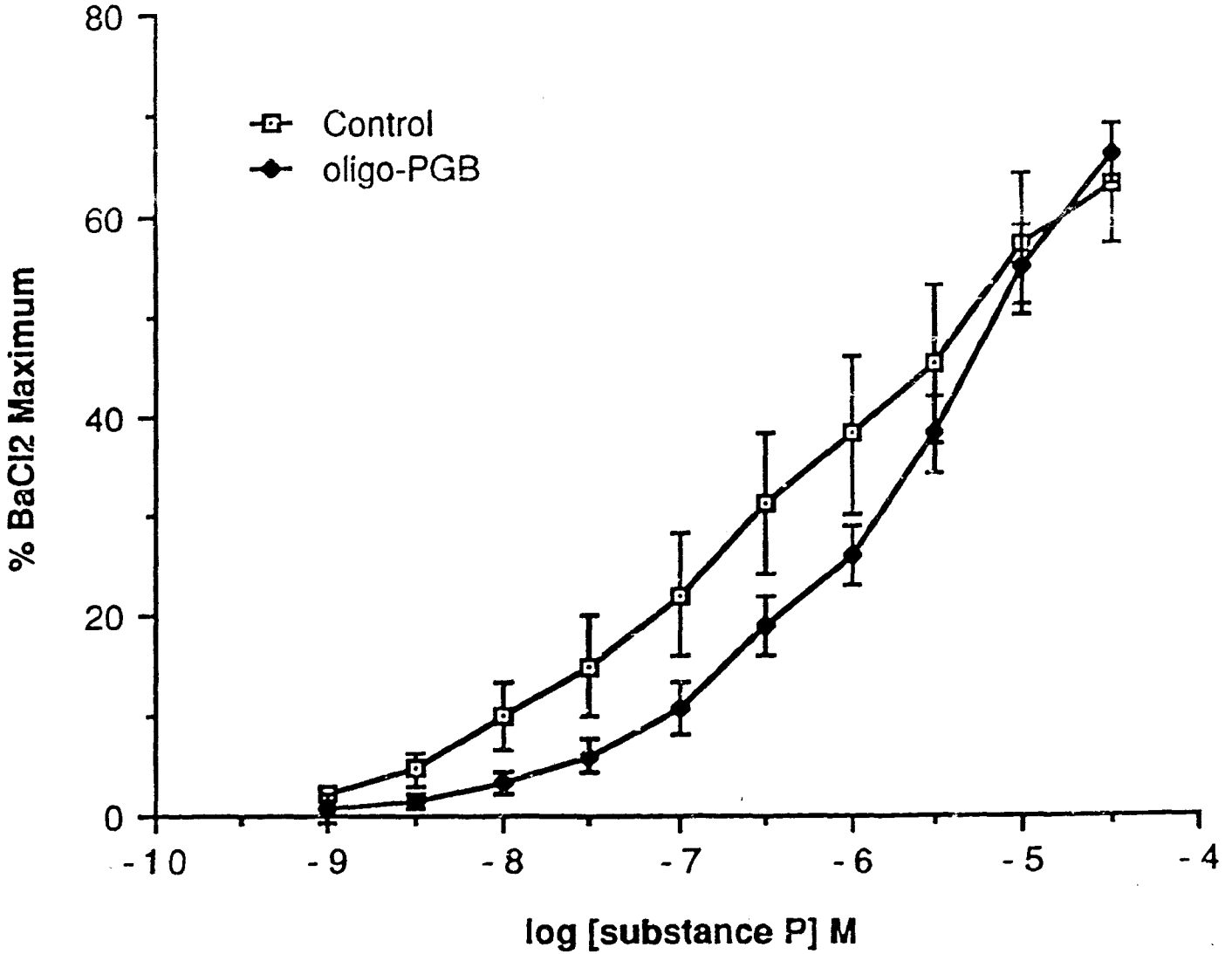

FIG.2 Dose response curve to substance P in presence (N=8) or in absence of oligo-PGB.

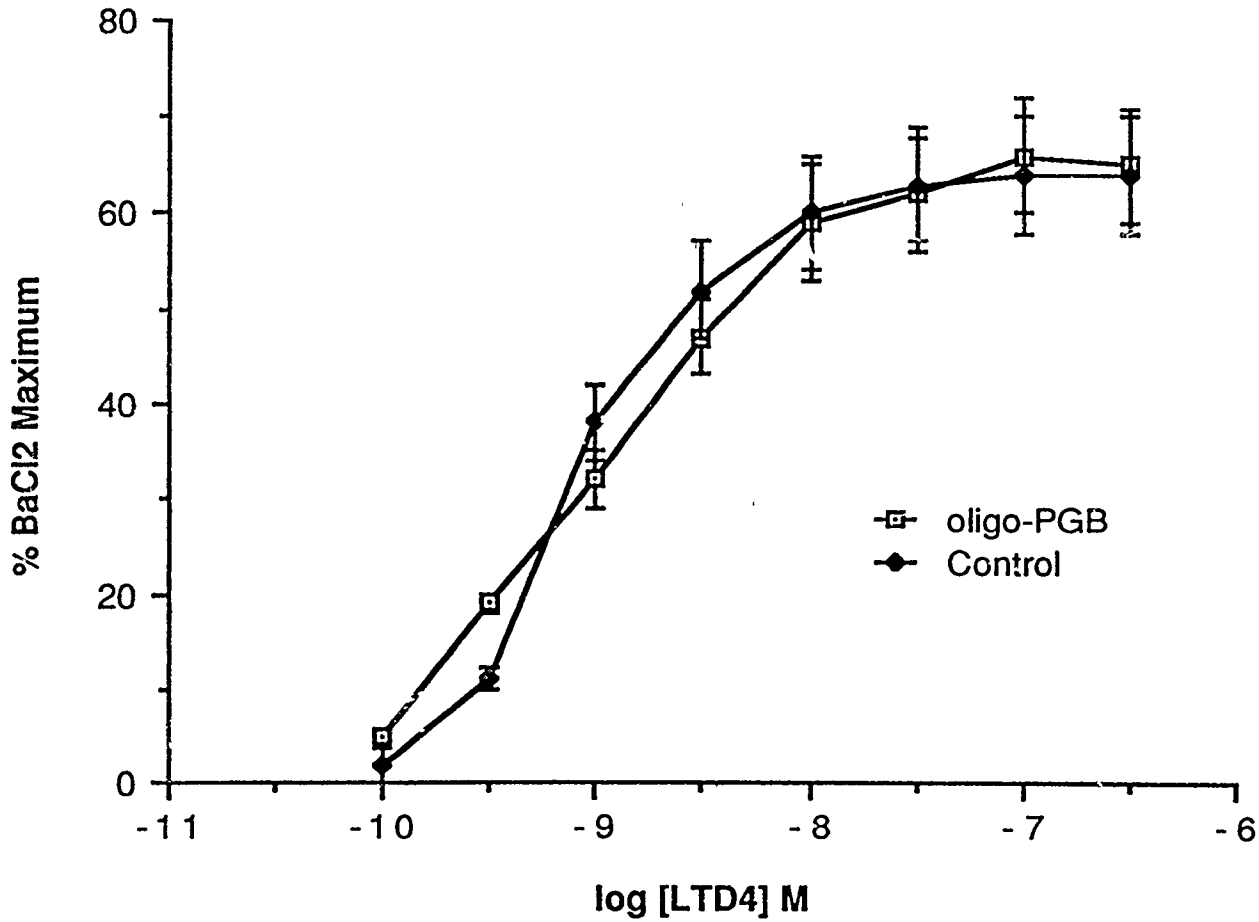

FIG.3 Dose response curve to LTD4 in the presence (N=4) or absence (N=4) of oligo-PGB.

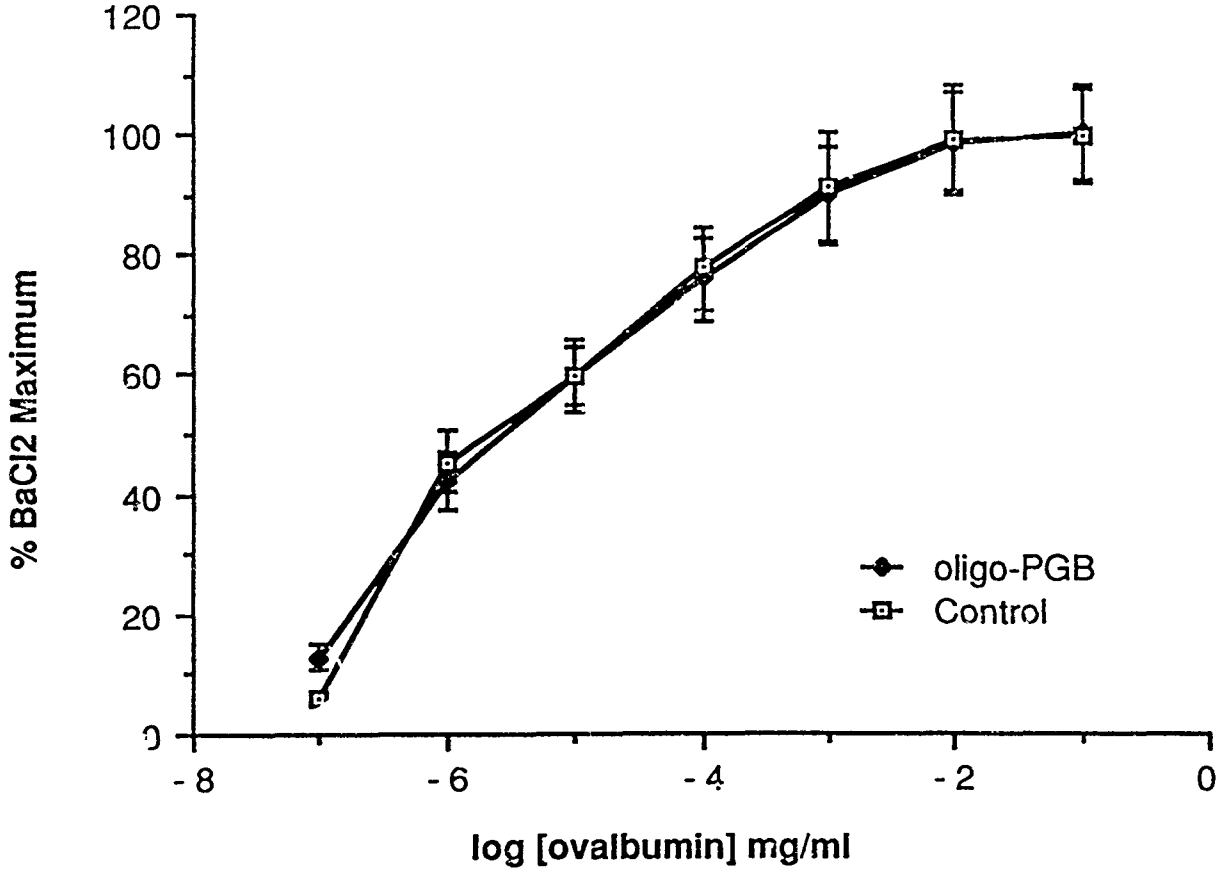

FIG.4 Dcse response curve to ovalbumin in left bronchi isolated from sensitized guinea pigs, in the presence of indomethacin $5X10^{-6}$ M. Oligo-PGB (10^{-5} M) was added 20 minutes before. N=6 in both groups.

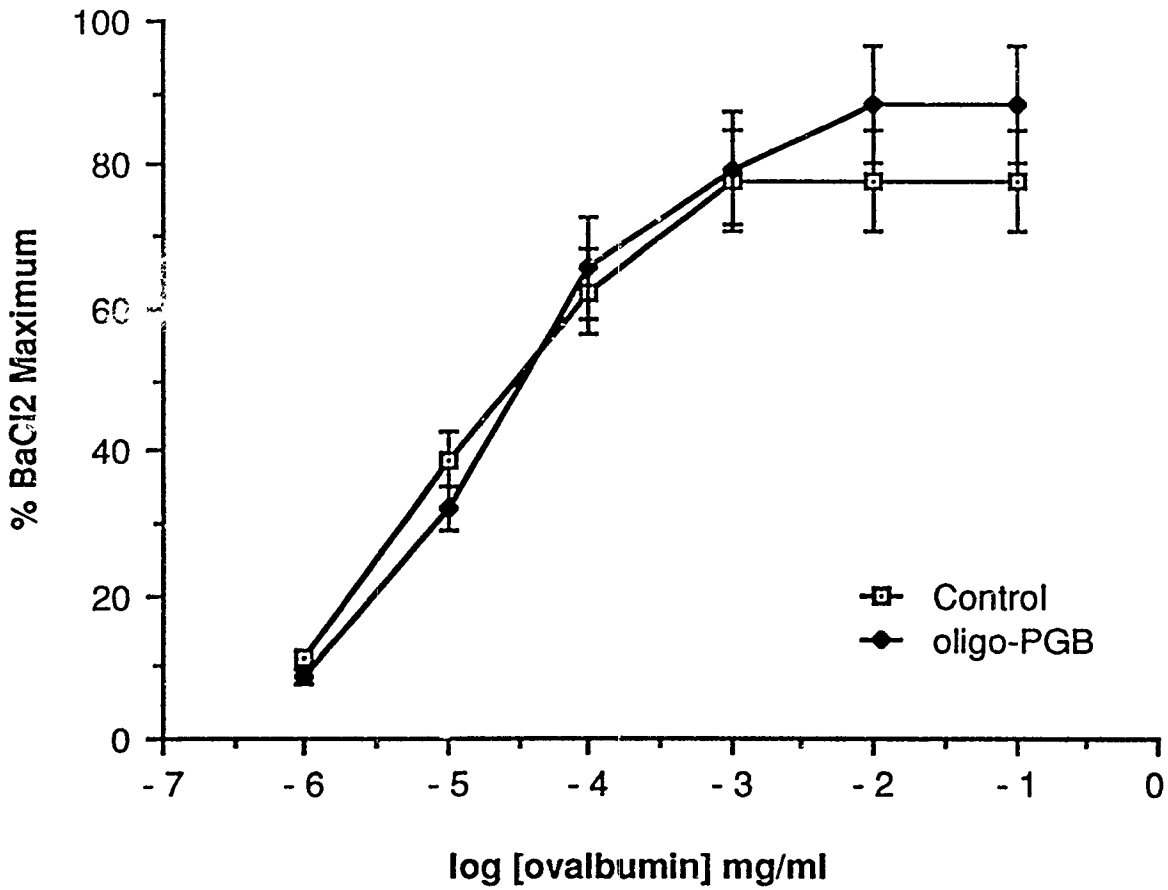

FIG.5 Dose response curve to ovalbumin in left bronchi isolated from sensitized guinea pigs and incubated for 18 hours in RPMI 1640 media with or without oligo-PGB (10^{-5} M). N=4 in both groups.

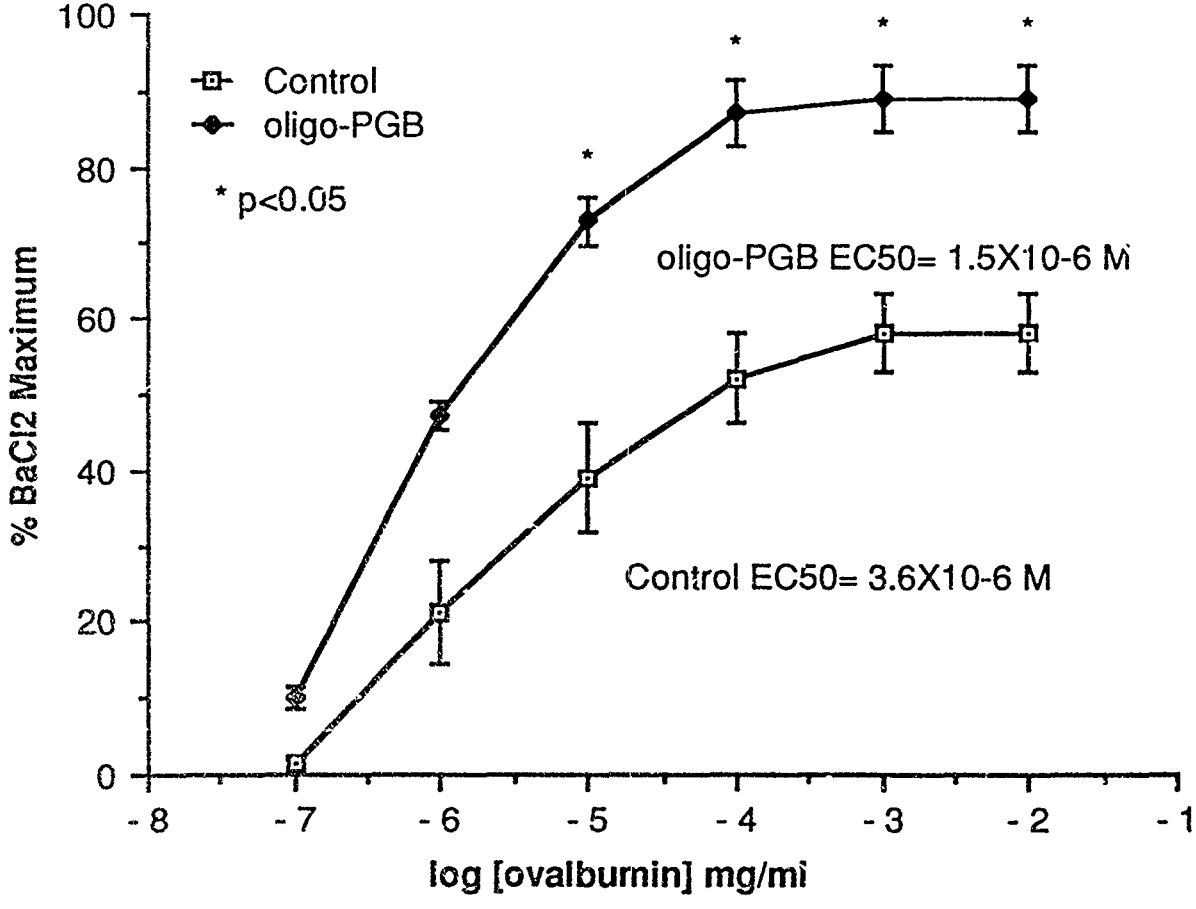

FIG.6 Dose response to ovalbumin in left bronchi isolated from sensitized guinea pigs and incubated for 24 hours in RPMI 1640 media with or without oligo-PGB (10^{-5} M). N=8 in both groups.

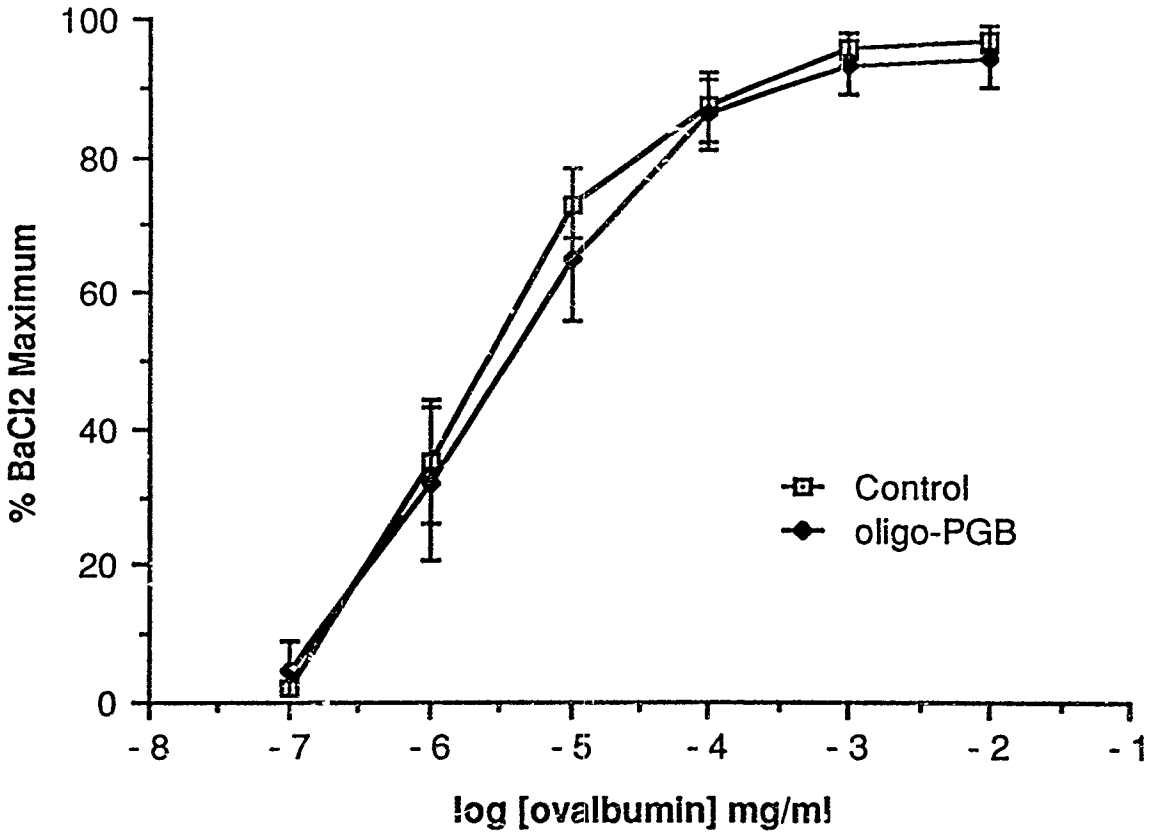

FIG.7 Dose response curve (DRC) to ovalbumin in left bronchi isolated from sensitized guinea pigs and incubated for 24 hours in RPMI 1640 media with or without oligo-PGB (10^{-5} M). Indomethacin $5X10^{-6}$ M was added 2 hours before the DRC. N=8 in both groups.

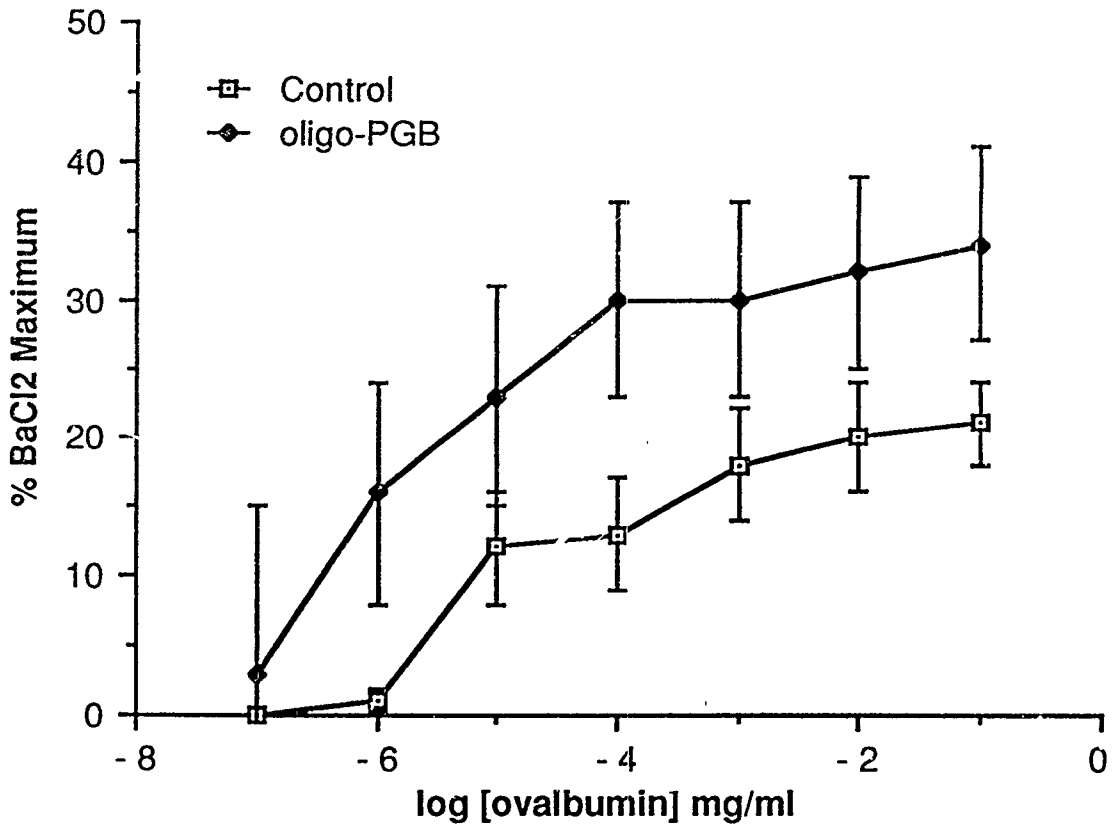

FIG.8 Dose response curve (DRC) to ovalbumin in left bronchi isolated from sensitized guinea pigs and incubated for 24 hours in RPMI 1640 media with or without oligo-PGB (10^{-5} M). Pyrilamine 10^{-6} M were added to the tissue bath 20 minutes before DRC. N=4 in both groups.

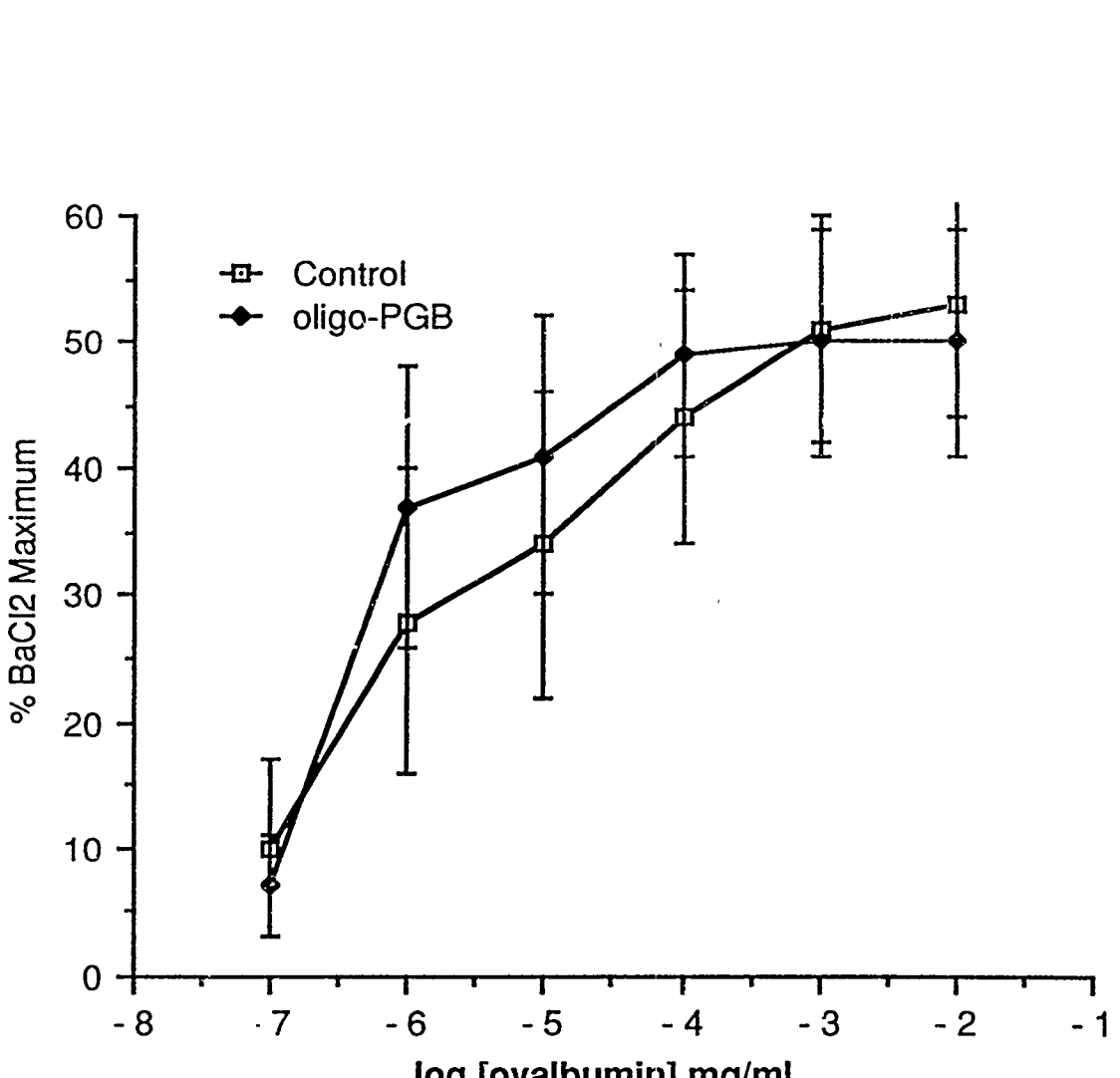

FIG.9 Dose response curve (DRC) to ovalbumin in left bronchi isolated from sensitized guinea pigs and incubated for 24 hours in RPMI 1640 media with or without oligo-PGB (10^{-5} M). Indomethacin $5X10^{-6}$ M and pyrilamine 10^{-6}M were added to the tissue bath. N=8 in both groups.